

WHAT IS CLAIMED IS:

1. A method comprising sequentially contacting a sample with at least a first stationary phase and a second stationary phase under chromatographic conditions, wherein the specificity of said first stationary phase for at least one constituent present in said sample is at least uncertain, and the specificity of said second stationary phase for said at least one constituent is certain, to at least determine the binding identity of said at least one constituent.
2. The method of Claim 1, wherein said method is a method of evaluating the specificity of said first stationary phase for said at least one constituent present in said sample.
3. The method of Claim 2, wherein said sample is contacted with said first stationary phase and then said second stationary phase.
4. The method of Claim 3, wherein said method further comprises:
  - (a) contacting said sample with said first stationary phase to bind a fraction of said sample that comprises said at least one constituent;
  - (b) separating said binding fraction from said first stationary phase; and
  - (c) contacting said binding fraction with said second stationary phase.
5. The method of Claim 3, wherein said first stationary phase comprises a pharmaceutical agent and said method is a method of determining the specificity of said pharmaceutical agent for said at least one constituent present in said sample.
6. The method of Claim 1, further comprising analyzing any constituents that did not bind to said second stationary phase.
7. The method of Claim 6, wherein said analyzing comprises using at least one of: one dimensional gel electrophoresis, two dimensional gel electrophoresis, matrix assisted laser desorption/ionization mass spectroscopy, liquid chromatography/mass spectroscopy,

biomolecular interaction, immunochemical analysis, nuclear magnetic resonance and circular dichroism.

8. The method of Claim 1, wherein said method is a method of determining the specificity of a pharmaceutical agent for at least one constituent present in said sample.

9. The method of Claim 8, wherein said sample is contacted with said second stationary phase first and said first stationary phase second.

10. The method of Claim 9, wherein said method further comprises:

- (a) contacting said sample with said second stationary phase to bind a fraction of said sample that comprises at least one constituent;
- (b) separating said binding fraction from said second stationary phase; and
- (c) contacting said binding fraction with said first stationary phase.

11. The method of Claim 9, wherein said first stationary phase comprises said pharmaceutical agent.

12. The method of Claim 10, further comprising analyzing any constituents that did not bind to said first stationary phase.

13. The method of Claim 12, wherein said analyzing comprises performing at least one of: one dimensional gel electrophoresis, two dimensional gel electrophoresis, matrix assisted laser desorption/ionization mass spectroscopy, liquid chromatography/mass spectroscopy, biomolecular interaction, immunochemical analysis, nuclear magnetic resonance and circular dichroism.

14. The method of Claim 1, wherein said sample comprises a population of proteins and said method is a method of separating a sub-population of proteins from said population of proteins.

15. The method of Claim 14, wherein said separating comprises:
  - (a) contacting said sample comprising a class of proteins with said first stationary phase to bind a fraction of said sample that comprises said population of proteins;
  - (b) collecting said binding fraction from said first stationary phase; and
  - (c) contacting said binding fraction with said second stationary phase so that said sub-population of proteins binds to said second stationary phase and any remaining members of said population do not bind to said second stationary phase.
16. The method of Claim 1, wherein said first and second stationary phases comprise ligands chosen from: antibodies or binding fragments thereof, diabodies, minibodies, antigens, dyes, single chain variants, proteins, glycoproteins, peptides, nucleic acids, vitamins, inorganic chemicals and organic chemicals.
17. The method of Claim 16, wherein at least one stationary phase comprises chlorotriazine affinity ligands.
18. The method of Claim 16, wherein at least one stationary phase comprises immunoaffinity ligands.
19. The method of Claim 16, wherein said first stationary phases comprises chlorotriazine affinity ligands and said second stationary phase comprises immunoaffinity ligands.
20. The method of Claim 1, wherein said first and second stationary phases are connected by a conduit.
21. A method of determining the specificity of a pharmaceutical agent for at least one constituent present in said sample comprising sequentially contacting a sample with at least a first stationary phase and a second stationary phase under chromatographic conditions, wherein the specificity of said first stationary phase for at least one constituent present in said sample is at least uncertain, and the specificity of said second stationary phase for said

at least one constituent is certain, to at least determine the binding identity of said at least one constituent.

22. The method of Claim 21, wherein said sample is contacted with said second stationary phase first and said first stationary phase second.

23. The method of Claim 22, wherein said method further comprises:

- (a) contacting said sample with said second stationary phase to bind a fraction of said sample that comprises at least one constituent;
- (b) separating said non-binding fraction from said second stationary phase; and
- (c) contacting said non-binding fraction with said first stationary phase.

24. The method of Claim 22, wherein said first stationary phase comprises said pharmaceutical agent.

25. The method of Claim 23, further comprising analyzing any constituents that did not bind to said first stationary phase.

26. The method of Claim 25, wherein said analyzing comprises performing at least one of: one dimensional gel electrophoresis, two dimensional gel electrophoresis, matrix assisted laser desorption/ionization mass spectroscopy, liquid chromatography/mass spectroscopy, biomolecular interaction, immunochemical analysis, nuclear magnetic resonance and circular dichroism.

27. A method of evaluating the specificity of a stationary phase comprising contacting a sample with at least a first stationary phase and second stationary phase under chromatographic conditions, wherein the specificity of said first stationary phase for at least one constituent present in the sample is at least uncertain, and the specificity of said second stationary phase for said at least one constituent is certain.

28. A method comprising forwarding data representing a result of an analysis step obtained by at least one of the method of Claim 6, the method of Claim 12 and the method of claim 25.

29. The method according to Claim 28, wherein said data is transmitted to a remote location.
30. A method comprising receiving data representing a result of an analysis step obtained by the method of Claim 28.
31. A system comprising:
- (a) a sample comprising at least one constituent;
  - (b) a first stationary phase wherein the specificity for at least one constituent present in said sample is at least uncertain; and
  - (c) a second stationary phase wherein the specificity for said at least one constituent is certain.
32. The system of Claim 31, wherein at least one of said stationary phases comprises chlorotriazine affinity ligands.
33. A device comprising a first stationary phase of uncertain specificity for at least one constituent of a sample and a second stationary phase of certain specificity for said at least one constituent of a sample.
34. The device of Claim 33, wherein said device is a chromatography column.
35. The device of Claim 33, wherein said device is a microfluidic device.
36. A kit comprising:
- (a) a first stationary phase of at least uncertain specificity;
  - (b) a second stationary phase of certain specificity; and
  - (c) instructions for using the first and second stationary phases in the method of Claim 1.